This presentation does not contain any proprietary, confidential, or otherwise restricted information





**BIOENERGY TECHNOLOGIES OFFICE** 

DOE BioEnergy Technologies Office (BETO) 2023 Project Peer Review

## **ABF Future Strategy – Implementation Plan**

April 4, 2023 Conversion Technologies

Nathan J. Hillson Berkeley Lab





# **Project Overview**

## **Project History**

- In September 2022, BETO instructed ABF strategic planning
  - Included guidance regarding the process, timeline, and representative deliverables to focus the process
- In December 2022, the ABF completed its strategic plan
  - Minor updates to the strategic plan were made through February 2023
- Goals and deliverables were included in the strategic plan
  - Milestones were moved to the implementation plan
- In March 2023, the ABF completed its implementation plan
  - Milestones were included
- In April 2023, the ABF received feedback at BETO Peer Review
  - (Now) concerning its implementation plan





## **Project Goal and BETO Relevance**

- Goal: Design a task structure that will achieve ABF's strategic goals, including annual milestones, that satisfies budget constraints and realizes as best possible the established FY25 deliverables
- Relevance to BETO's goals: This activity ensures that ABF planned activities for FY23-25 meet BETO's budget allocation for the ABF and are consistent with a path likely to accomplish the deliverables established in the BETO-approved revised ABF strategic plan
- Specific research question: How to develop a budget-constrained implementation of a strategic plan that is likely to accomplish established deliverable requirements, and gracefully breaks from the status quo to preserve as best possible capabilities and relationships







# 1 – Approach

## **Technical Approach**

- Follow BETO's guidance for ABF implementation planning
  - Akin to BETO's guidance for strategic planning
- Use ABF implementation planning working groups' input
  - reviewing history, baselining, industry, culture, partnering (continued from strategic planning process)
- Use inclusive implementation planning tools and processes
  - Miro for online real-time collaborative ideation and refinement
  - Implementation planning process templates
- Divide and conquer work across goal areas
  - Define clear accountability and responsibility for activity description, resourcing, and milestone development
- Use small / focused groups to make progress as needed
  - Straw-person proposals may rapidly advance work when a large inclusive group is converging slowly
- Request feedback from BETO leadership and ABF advisors
  - Make corresponding adjustments as needed, including those suggested at BETO Peer Review



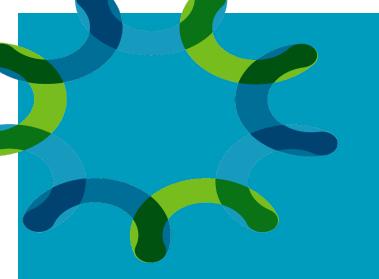


## Top Potential Challenges

- How to design tasks that will achieve the strategic goals
- Maintain morale and relationships while navigating the transition from imagining what could be to determining what will be
  - And, what will not be
- Developing a transparent, inclusive, and equitable process for budget-constrained activity prioritization
  - While where necessary to make progress and maintain relationships, some deviations are likely required
  - Balance contrasting opinions and select from multiple viable approaches
  - Deliverables were intentionally developed so as not to overly constrain how they might be accomplished
- Support broadly enabling capabilities and hosts
  - That are not directly contributing to prioritized sustainable aviation fuel or biochemicals activities
  - But that have historically been, or are anticipated to be, requested for use in collaboration projects







# 2 - Progress and Outcomes

## Progress made towards project goal

- Activities resourced across ABF goals, within budget allocation
  - Partnerships: Actively develop funds-in and BETO-funded projects that result in technology transfer to industry
  - Tools: Develop and demonstrate engineering biology, computational, and bioprocess tools that advance strain performance for the sustainable production of fuels and chemicals
  - Sustainable Aviation Fuels: Develop innovative pathways, strains, and processes for the production of sustainable aviation fuels
    - Only 2 SAF target/hosts to be pursued
  - Biochemicals: Develop innovative pathways, strains, and processes for the sustainable production of direct replacement and performance-advantaged chemicals
    - Only 2 biochemicals target/hosts to be pursued
- The following slides list key activities and milestones for each goal for FY23-25





## **Partnerships**

- Actively engage industry to develop and promote the adoption of ABF processes and technologies
  - Business development, market research (including customer discovery interviews, and surveys), and marketing
  - Establish ABF core-industry partnerships ( >20% cost-share)
- Develop new IP management plan to enable more efficient licensing
- Demonstrate and measure ABF's impact on and contributions to the biomanufacturing industry
  - ABF core-industry partnerships, funding opportunity partnerships, and funds-in partnerships

**Partnerships Goal**: Actively develop funds-in and BETO-funded projects that result in technology transfer to industry





## **Partnerships**

#### FY23 milestones

- Business development co-leads at NREL and LBL onboarded
- New ABF IP management plan developed

#### FY24 milestone

At least 2 funds-in projects established

#### FY25 milestones

- 50% of ABF's budget as funding opportunity partnerships and ABF core-industry partnerships achieved
- Report on ABF partnership activities submitted, including CO2e reduction potentials, partner testimonials regarding commercialization time and cost savings, and extents of ABF funds-in support
- 5% of ABF's annual budget achieved as funds-in support

**Partnerships Goal**: Actively develop funds-in and BETO-funded projects that result in technology transfer to industry





## Tools - development and benchmarking

- Identify and prioritize capability gaps / opportunities
- Develop new capabilities
  - Machine learning tools to predict the temperature and pH optima of enzymes
  - Protein structural modeling to change substrate specificity and improve thermostability
  - New automation onboarding (Vantage and anaerobic Tecan)
  - Automation of transformation through omics sample prep (includes high-throughput electroporation/conjugation)
  - Genome-scale screening (e.g., RB-TnSeq) and genome-modification tools (e.g., SAGE, CRISPR)
  - Isotopomer/genome fluxomics/metabolic flux analysis models, and multi-labeled substrate methods
  - Expanded targeted metabolomics coverage of key pathway molecules, substrates, and products
  - High-throughput high-quality proteomics methods (e.g., plate-based SPCE, DIA-MS)
  - High-throughput RNA-seq and data analysis pipeline
  - Data visualization tools
  - Integration of machine learning and mechanistic models, and machine learning to improve flux model predictions
- Sunset capability development
- Capability benchmarking
- Monitor capability capacities, usage, and transfer to industry
- Maintain and continuously develop capabilities

**Tools Goal**: Develop and demonstrate engineering biology, computational, and bioprocess tools that advance strain performance for the sustainable production of fuels and chemicals





## Tools – host onboarding and development

- Strategically assess the prospective onboarding of additional hosts
  - Focus on expanding the metabolic, phylogenetic, and bioprocess diversity available within the ABF
  - Anticipate future needs including use in sustainable aviation fuels and biochemicals goal activities
- Use market pull to direct host development beyond onboarding
  - ABF core-industry partnerships, funds-in projects
  - Sustainable aviation fuels and biochemicals goal activities
- Demonstrate how extended host diversity enhances process performance and industry engagement
- Update the host Tier system as needed

**Tools Goal**: Develop and demonstrate engineering biology, computational, and bioprocess tools that advance strain performance for the sustainable production of fuels and chemicals





### Tools

#### FY23 milestones

- Go/No-Go: Colony identities post FACS match distribution profile expectations
- New automation (Vantage, anaerobic Tecan) onboarded
- Pilot benchmarking experiment initiated
- Machine learning method for enzyme temperature and pH optima predictions developed
- Genome-scale screening tool (e.g. RB-TnSeq) successfully constructed in a production strain, and actionable outcomes for strain improvement reported

#### FY24 milestones

- Automated transformation through omics sample prep demonstrated for 2 organisms
- Two additional benchmarking experiments completed
- Additional genome-scale screening tool (e.g. over-expression library) successfully constructed in a production strain, and actionable outcomes for strain improvement reported

#### FY25 milestones

- At least 6 new technologies across Design (including TEA/LCA), Build, Test (including scale-up), and Learn demonstrated between FY23 and FY25
- Operational performance gains of significant impact demonstrated for at least 2 ABF benchmarked technologies
- Report delivered demonstrating assessments of ABF tools against benchmarks, the ABF capacity for using each tool, frequencies of ABF capabilities usage in internal and partner projects, and tool transfers to industry
- Report delivered demonstrating transfer of host onboarding and development efforts to industrial partners

**Tools Goal**: Develop and demonstrate engineering biology, computational, and bioprocess tools that advance strain performance for the sustainable production of fuels and chemicals





## Sustainable aviation fuels – ethanol

- Leverage existing and develop as needed process models for ABF pathways and conduct TEA and LCA
- Use an anaerobic, cellulolytic thermophile to deconstruct biomass polysaccharides
  - In the absence of thermochemical pretreatment and enzymatic hydrolysis (i.e. consolidated bioprocessing -CBP) to produce the SAF intermediate ethanol
- Leverage the core capabilities of the ABF to advance the TRL for this CBP concept
- Strain onboarding and engineering
  - Procure Caldicellulosiruptor strains from culture collections and test their efficacy degrading both whole corn stover and residual polysaccharides from lignin-first biorefinery processes
  - Onboard the selected strain based on biomass solubilization extents, projected ethanol production capacity, etc.
  - Deploy genetic tools, and conduct systems biology baseline studies
- Process development and scale-up
  - Optimize media for the selected host to reach high optical density values
  - Develop fermentation approaches that enable high optical density on whole biomass, and a metabolomics effort to understand metabolic pathways in the presence of whole biomass





### Sustainable aviation fuels – ethanol

#### FY23 milestones

- Go/No-Go: Consolidated bioprocessing of biomass to ethanol exhibits sufficient commercial interest to pursue
- All accessible Caldicellulosiruptor strains (and other relevant microbes) procured from publicly-available culture collections
- Biomass solubilization trials conducted in temperature and pH-controlled conditions on whole corn stover and lignin-extracted biomass substrates (in collaboration with Lignin-First Biorefinery Development project)

#### FY24 milestones

- Down-selection to a single CBP strain made
- At least 2 off-target pathways knocked-out
- Media optimization conducted on corn stover and/or lignin-first substrates, and the biomass solubilization quantified as a function of solids loadings

#### FY25 milestones

- 60% of theoretical ethanol yield produced at 10 wt% solids loading
- Limitations for improving solids loadings identified (to be addressed in FY26-28)





## Sustainable aviation fuels – alkane

- Leverage existing and develop as needed process models for ABF pathways and conduct TEA and LCA
- Establish baseline titer, rate, and yield of key biomolecules leveraging existing data and expertise
  - For one of: TAGs for HC-HEFA, farnesene for SIP, TAGs/fatty acids/esters for HEFA, CHJ, or co-processing

#### Strain onboarding and engineering

- Map out process scenarios for lipid/alkane-based SAFs, screen and select host, identify engineering strategies
- Baseline production (titer, rate, yield) for key biomolecules
- Identify biosynthetic routes to SAF targets, select hosts for testing, design and build strains, and optimize TRY to meet at least 50% GHG emissions reductions relative to fossil-based jet fuel at pilot scale
- Establish engineering tools needed to enable efficient engineering
- Identify new host engineering targets to improve TRY through rational and AI/ML guided efforts

#### Process development and scale-up

- Conduct bioreactor cultivations and optimize conditions to maximize titers on relevant feedstocks
- Examine specific scenarios for standalone microbial SAF technology or co-integrated technology where microbial alkane production is combined with existing sources of fats and alkanes
- Collaborate with the Separations Consortium to recover secreted hydrophobic products using Zaiput systems.





## Sustainable aviation fuels – alkane

#### FY23 milestone

- Go/No-Go: Down select to one alkenes process to pursue in FY24 and beyond
- Go/No-Go: Down-selected process for alkenes exhibits sufficient commercial interest to pursue

#### FY24 milestones

 Baseline TRY established for alkane sustainable aviation fuel (oils, free fatty acids, and alcohols, and terpenes for HEFA, CHJ, HC-HEFA, SIP, and co-processing)

#### FY25 milestone

 Lipid or alkane production achieved in an oleaginous microbe at 75% of theoretical yield aided by in situ product recovery





### Biochemicals – muconate

- Leverage existing and develop as needed process models for ABF pathways and conduct TEA and LCA
- Reach industrially relevant titers, rates, and yields with a specific focus on technology transfer
- Conduct work on muconic acid as part of a core-industry partnership with in-kind cost share
  - Provided by scale-up partners and companies interested in bio-based polyamide and polyester production

#### Strain engineering

- To increase process metrics (TRY) further, need to explore new lines of investigation:
- Finalize efforts to on-board arabinose utilization into muconic acid-producing strains
- Demonstrate genome-scale tools, including RB-TnSeq and overexpression libraries to discover new strategies to increase titer, rate, and yield for muconic acid
- Optimize media optimization with the ART tool

#### Process development and scale-up

- Conduct bioprocess development at the 0.5-1,000 L scale in close concert with Test and Learn activities
- Produce muconic acid for downstream processing and performance testing
- Work in close concert with the Separations Consortium to conduct in situ product recovery of muconic acid using weak anion exchange resins





### Biochemicals – muconate

#### FY23 milestones

- Go/No-Go: Muconic acid from hydrolysate sugars exhibits sufficient commercial interest to pursue
- Arabinose utilization engineered into muconic acid-producing strains of *P. putida* and the resulting improved muconic acid productivity demonstrated
- Media optimized for a muconic acid-producing P. putida strain with the ART tool

#### FY24 milestone

 Muconic acid production scaled-up to 100 L to produce materials for downstream processing and performance testing with industrial partners

#### FY25 milestones

- Muconic acid-producing strains and bioprocesses transferred to industry to produce direct replacement and performance-advantaged chemicals at industrially relevant titers, rates, and yields and at 70% reduction in GHG emissions relative to fossil-based production
- Clear path to commercialization for muconic acid by 2030 demonstrated





## Biochemicals – 3-hydroxypropionic acid

- Leverage existing and develop as needed process models for ABF pathways and conduct TEA and LCA
- Reach industrially relevant titers, rates, and yields with a specific focus on technology transfer
- Connect work on 3HP to a core-industry partnership with in-kind cost share
  - Provide to companies interested in bio-based acrylate, polyester, and other bioplastic production
- Strain engineering
  - Engineer additional targets from Test-Learn to improve TRY metrics determined by TEA/LCA
  - Assess current and future targets for A. niger aspartate routes and R. toruloides mal-CoA routes
- Process development and scale-up
  - Perform media and bioprocess condition optimization in close coordination with Test / Learn to increase TRY and minimize nutrient inputs at relevant pH determined from TEA/LCA and downstream product purity perspectives
  - Conduct scale-up and scale-down experiments from BioLector to 20-300 L
  - Work in close concert with the Separations Consortium to conduct in situ product recovery of 3HP using weak anion exchange resins





## Biochemicals – 3-hydroxypropionic acid

#### FY23 milestones

- Go/No-Go: Down select to one 3HP process to pursue in FY24 and beyond
- Go/No-Go: Down-selected process for 3HP exhibits sufficient commercial interest to pursue
- 3HP production optimized to >40 g/L on relevant feedstocks

#### FY24 milestone

- Media optimized for a 3HP-producing strain with the ART tool
- New host engineering targets identified to improve TRY through rational and AI/ML guided efforts

#### FY25 milestones

- 3HP acid-producing strains and bioprocesses transferred to industry to produce direct replacement and performance-advantaged chemicals at industrially relevant titers, rates, and yields and at 70% reduction in GHG emissions relative to fossil-based production
- Clear path to commercialization for 3HP by 2030 demonstrated



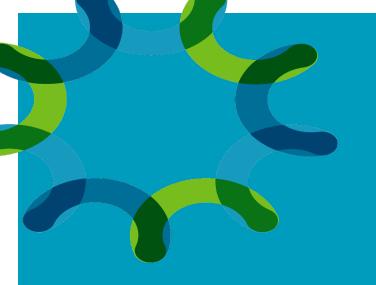


## **Risks and Mitigations**

Risk	Severity	Description	Mitigation Plan
Unable to secure funds-in projects	Medium	Business development efforts fail to yield funds-in projects	Suggest changes to ABF tasks to better align with industry's needs and relay contracting process issues
Core unable to secure cost-share	Medium	At the end of FY25, \$5M/year of core activities (one third) will need to have 20% cost share	Fall-back position is reallocating core resources to funding opportunities
Benchmarking doesn't demonstrate significant impact	Medium	Depending on how benchmarking experiments designed, and what the results are, there may not be indications of significant impact	The ABF ExComm with BETO concurrence could repeat benchmarking before making sunsetting determination
SAF/Biochemicals focus jeopardizes capabilities	High	Prioritizing SAF and biochemicals could jeopardize keeping ABF capabilities cutting edge for industry projects	Use ABF funding opportunities and business development/funds-in projects to support capability building activities
Caldicellulosiruptor not able to reach high cell density	High	Caldicellulosiruptor has not been reported to reach high optical densities when growing in laboratory cultures to date	Media development to achieve higher cell densities. Investigate the biomass growth limitations through knockouts
SAF/Biochemical projects fail to resonate w/ industry	Medium	The SAF and biochemicals processes selected by the ABF may not be the most relevant to industry	Go/no-go commercial interest stage gates for all SAF and biochemicals projects at the end of FY23







# 3 – Impact

### Impact on state of technology/industry if successful

#### A re-imagined DOE Agile BioFoundry resumes operations

Renewed focus on industry partnerships, sustainable aviation fuels, and renewable biochemicals

#### Industry-partnered commercialization paths

- At least 50% GHG emissions reductions and paths to economic viability for thermophilic ethanol and microbial alkanes to SAF processes
- At least one biochemical process with at least 70% GHG emissions reductions with a commercial partner and a path to commercialization by 2030
- At least 2 funds-in projects
- Enable at least 500 kT CO2e reduction through commercial partnership projects

### ABF-developed capability benchmarking

 At least 2 ABF capabilities compared to best industry-accessible alternative benchmarks demonstrate operational performance gains of significant impact





## **Summary**

- Goal: Design a task structure that will achieve ABF's strategic goals, including annual milestones, that satisfies budget constraints and realizes as best possible the established FY25 deliverables
- Relevance to BETO's goals: This activity ensures that ABF planned activities for FY23-25 meet BETO's budget allocation for the ABF and are consistent with a path likely to accomplish the deliverables established in the BETO-approved revised ABF strategic plan
- Outcomes: Developed a budget-constrained implementation plan that largely accomplishes then deliverables established in the ABF strategic plan, and gracefully breaks from the status quo to preserve as best possible capabilities and relationships





## **Quad Chart Overview**

#### **Timeline**

• Project start: October 1, 2022

Project end: September 30, 2025

#### **Project Goal**

Develop biomanufacturing tools, processes, and partnerships that enable sustainable industrial production of renewable fuels and chemicals for the nation

#### **End of Project Milestone**

- Transfer strains and bioprocesses for SAF and biochemicals to industry at industrially relevant titers, rates, and yields and at 70% reduction in GHG emissions relative to fossil-based production.
- Achieve 5% of ABF's annual budget as funds-in support, and based on learnings, strategize paths to future expansion
- Demonstrate at least 6 new technologies across Design (including TEA/LCA), Build, Test (including scale-up), and Learn

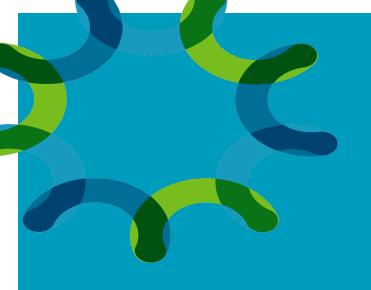
## Funding Mechanism AOP

#### **Project Partners**

LBNL (23%), SNL (20%), NREL (18%), PNNL (17%), LANL (8%), ORNL (8%), ANL (6%)







# **Additional Slides**

#### Publications, Patents, Presentations, Awards, and Commercialization

#### 70 publications, 189 presentations to date

- 54 publications and 115 presentations since FY2019
- The following slides list these publications and presentations

#### 17 patents, 10 records of invention, 9 software disclosures, & 2 licenses

• The following slides list these intellectual property assets





- Garima Goyal, Zak Costello, Jorge Alonso Guitierrez, Aram Kang, Taek Soon Lee, Hector Garcia Martin, and Nathan J Hillson. (2018) "Parallel Integration and Chromosomal Expansion of Metabolic Pathways" ACS Synthetic Biology DOI: 10.1021/acssynbio.8b00243
- Jha RK, Narayanan N, Pandey N, Bingen JM, Kern TL, Johnson CW, Strauss CEM, Beckham GT, Hennelly SP, Dale T. Sensor-enabled alleviation of product inhibition in chorismate pyruvate-lyase. ACS Synthetic Biology (2019), 10.1021/acssynbio.8b00465.
- Oyetunde, T., Liu, D., Martin, H. G., & Tang, Y. J. "Machine learning framework for assessment of microbial factory performance." PloS one 14.1: e0210558 (2019).
- Ando, D., and Garcia Martin, H. "Genome-Scale 13 C Fluxomics Modeling for Metabolic Engineering of Saccharomyces cerevisiae" Microbial Metabolomics. Humana Press, New York, NY: 317-345 (2019).
- Costello, Zak, and Hector Garcia Martin. "How to Hallucinate Functional Proteins." arXiv preprint arXiv:1903.00458 (2019).
- Chen Y, Vu J, Thompson MG, Sharpless WA, Chan LJG, et al. (2019) A rapid methods development workflow for high-throughput quantitative proteomic applications. PLOS ONE 14(2): e0211582. https://doi.org/10.1371/journal.pone.0211582





- Paul Opgenorth, Zak Costello, Takuya Okada, Garima Goyal, Yan Chen, Jennifer Gin, Veronica T. Benites, Markus de Raad, Trent R. Northen, Kai Deng, Samuel Deutsch, Edward E.K. Baidoo, Christopher J. Petzold, Nathan J Hillson, Hector Garcia Martin, and Harry R Beller. (2019) "Lessons from two Design-Build-Test-Learn cycles of dodecanol production in Escherichia coli aided by machine learning". ACS Synth. Biol., DOI: 10.1021/acssynbio.9b00020 https://pubs.acs.org/doi/10.1021/acssynbio.9b00020
- Nathan Hillson, Mark Caddick, Yizhi Cai, Jose A. Carrasco, Matthew Wook Chang, Natalie C. Curach, David J. Bell, Rosalind Le Feuvre, Douglas C. Friedman, Xiongfei Fu, Nicholas D. Gold, Markus J. Herrgård, Maciej B. Holowko, James R. Johnson, Richard A. Johnson, Jay D. Keasling, Richard I. Kitney, Akihiko Kondo, Chenli Liu, Vincent J. J. Martin, Filippo Menolascina, Chiaki Ogino, Nicola J. Patron, Marilene Pavan, Chueh Loo Poh, Isak S. Pretorius, Susan J. Rosser, Nigel S. Scrutton, Marko Storch, Hille Tekotte, Evelyn Travnik, Claudia E. Vickers, Wen Shan Yew, Yingjin Yuan, Huimin Zhao & Paul S. Freemont. (2019) "Building a global alliance of biofoundries". Nature Communications, 10:2040 https://doi.org/10.1038/s41467-019-10079-2



- Jesus F. Barajas, Ryan P. McAndrew, Mitchell G. Thompson, Tyler W. H. Backman, Bo Pang, Tristan de Rond, Jose H. Pereira, Veronica T. Benites, Héctor García Martín, Edward E. K. Baidoo, Nathan J. Hillson, Paul D. Adams, and Jay D. Keasling. (2019) "Structural insights into dehydratase substrate selection for the borrelidin and fluvirucin polyketide synthases" J Ind Microbiol Biotechnol https://doi.org/10.1007/s10295-019-02189-z
- Jesus F. Barajas, Maren Wehrs, Milton To, Lauchlin Cruickshanks, Rochelle Urban, Adrienne McKee, John Gladden, Ed-Been-Goh, Margaret E. Brown, Diane Pierotti, James M. Carothers, Aindrila Mukhopadhyay, Jay D. Keasling, Jeffrey L. Fortman, Steven W. Singer, Constance B. Bailey. (2019) "Isolation and characterization of bacterial cellulase producers for biomass deconstruction: A microbiology laboratory course" J Microbiology and Biology Education. Just accepted. https://doi.org/10.1128/jmbe.v20i2.1723
- Mitchel G. Thompson, Jacquelyn Blake-Hedges, Pablo Cruz-Morales, Jesus F. Barajas, Samuel C. Curran, Christopher B. Eiben, Nicholas C. Harris, Veronica T. Benites, Jennifer W. Gin, William A. Sharpless, Jose H. Pereira, Edward E.K. Baidoo, Christopher C. Petzold, Paul D. Adams, Adam P. Arkin, Adam M. Deutschbauer, Jay D. Keasling (2019) "Massively parallel fitness profiling reveals multiple novel enzymes in pseudomonas putida lysine metabolism" MBio. https://doi.org/10.1128/mBio.02577-18





- Chen, Yan; Guenther, Joel; Gin, Jennifer; Chan, Leanne Jade; Costello, Zak; Ogorzalek, Tadeusz; Tran, Huu; Blake-Hedges, Jacquelyn; Keasling, Jay D; Adams, Paul; Garcia Martin, Hector; Hillson, Nathan; Petzold, Christopher. (2019) "An automated 'cells-to-peptides' sample preparation workflow for high-throughput, quantitative proteomic assays of microbes" Journal of Proteome Research Manuscript ID: pr-2019-00455n Submitted July 8, 2019 Accepted 8/22/2019 https://doi.org/10.1021/acs.jproteome.9b00455
- Carbonell, P., Radivojevic, T. and Garcia Martin, H., 2019. Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation. ACS Synthetic Biology 1474-1477 (2019)
- Roell, G.W., Carr, R.R., Campbell, T., Shang, Z., Henson, W.R., Czajka, J.J., Martín, H.G., Zhang, F., Foston, M., Dantas, G. and Moon, T.S. A concerted systems biology analysis of phenol metabolism in Rhodococcus opacus PD630. Metabolic engineering (2019).
- Tijana Radivojevic, Elena Akhmatskaya, "Modified Hamiltonian Monte Carlo for Bayesian inference", Statistics and Computing, https://doi.org/10.1007/s11222-019-09885-x
- Pablo Carbonell, Tijana Radivojevic, Héctor García Martín "Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation", ACS Synth. Biol. 2019, 8, 1474?1477





- Gayle J. Bentley, Niju Narayanan, Ramesh K. Jha, Davinia Salvachúa, Joshua R. Elmore, George L. Peabody, Brenna A. Black, Kelsey Ramirez, Annette De Capite, William E. Michener, Allison Z. Werner, Dawn M. Klingeman, Heidi S. Schindel, Robert Nelson Lindsey Foust, Adam M. Guss, Taraka Dale, Christopher W. Johnson, Gregg T. Beckham, "Engineering glucose metabolism for enhanced muconic acid production in Pseudomonas putida KT2440", in press at Metabolic Eng.
- Peabody GL, Elmore JR, Martinez-Baird J, and Guss AM. Engineered Pseudomonas putida KT2440 co-utilizes galactose and glucose. Biotechnol Biofuels 12, 295 (2019) doi:10.1186/s13068-019-1627-0
- Christopher B. Eiben, Tristan de Rond, Clayton Bloszies, Jennifer Gin, Jennifer Chiniquy, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, Oliver Fiehn, Jay D. Keasling. (2019) "Mevalonate Pathway Promiscuity Enables Noncanonical Terpene Production", ACS Synth. Biol. https://doi.org/10.1021/acssynbio.9b00230
- Yan Chen, Deepwanita Banerjee, Aindrila Mukhopadhyay, Christopher J. Petzold. (2020)
   "Systems and synthetic biology tools for advanced bioproduction hosts", Curr. Op. Biotechnol. https://doi.org/10.1016/j.copbio.2019.12.007





- Jacquelyn M. Blake-Hedges, Jose Henrique Pereira, Pablo Cruz-Morales, Mitchell G.
  Thompson, Jesus F. Barajas, Jeffrey Chen, Rohith N. Krishna, Leanne Jade G. Chan, Danika
  Nimlos, Catalina Alonso-Martinez, Edward E. K. Baidoo, Yan Chen, Jennifer W. Gin, Leonard
  Katz, Christopher J. Petzold, Paul D. Adams, Jay D. Keasling. (2019) "Structural Mechanism
  of Regioselectivity in an Unusual Bacterial Acyl-CoA Dehydrogenase", J. Am. Chem. Soc.
  https://doi.org/10.1021/jacs.9b09187
- Thompson, Mitchell G., Allison N. Pearson, Jesus F. Barajas, Pablo Cruz-Morales, Nima Sedaghatian, Zak Costello, Megan E. Garber et al. "Identification, characterization, and application of a highly sensitive lactam biosensor from Pseudomonas putida." ACS Synthetic Biology (2019).
- Production of ent-kaurene from lignocellulosic hydrolysate in Rhodosporidium toruloides.
   Geiselman GM, Zhuang X, Kirby J, Tran-Gyamfi MB, Prahl JP, Sundstrom ER, Gao Y, Munoz Munoz N, Nicora CD, Clay DM, Papa G, Burnum-Johnson KE, Magnuson JK, Tanjore D, Skerker JM, Gladden JM. Microb Cell Fact. 2020 Feb 5;19(1):24.





- Chen, Yan; Guenther, Joel; Gin, Jennifer; Chan, Leanne Jade; Costello, Zak; Ogorzalek, Tadeusz; Tran, Huu; Blake-Hedges, Jacquelyn; Keasling, Jay D; Adams, Paul; Garcia Martin, Hector; Hillson, Nathan; Petzold, Christopher. (2019) "An automated 'cells-to-peptides' sample preparation workflow for high-throughput, quantitative proteomic assays of microbes" Journal of Proteome Research Manuscript ID: pr-2019-00455n Submitted July 8, 2019 Accepted 8/22/2019 https://doi.org/10.1021/acs.jproteome.9b00455
- Carbonell, P., Radivojevic, T. and Garci?a Marti?n, H., 2019. Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation. ACS Synthetic Biology 1474-1477 (2019)
- Roell, G.W., Carr, R.R., Campbell, T., Shang, Z., Henson, W.R., Czajka, J.J., Martín, H.G., Zhang, F., Foston, M., Dantas, G. and Moon, T.S. A concerted systems biology analysis of phenol metabolism in Rhodococcus opacus PD630. Metabolic engineering (2019).
- Pablo Carbonell, Tijana Radivojevic, Héctor García Martín "Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation", ACS Synth. Biol. 2019, 8, 1474?1477





- Gayle J. Bentley, Niju Narayanan, Ramesh K. Jha, Davinia Salvachu?a, Joshua R. Elmore, George L. Peabody, Brenna A. Black, Kelsey Ramirez, Annette De Capite, William E. Michener, Allison Z. Werner, Dawn M. Klingeman, Heidi S. Schindel, Robert Nelson, Lindsey Foust, Adam M. Guss, Taraka Dale, Christopher W. Johnson, Gregg T. Beckham. Engineering glucose metabolism for enhanced muconic acid production in Pseudomonas putida KT2440. Metabolic Engineering (2020), 59, 64-75
- Peabody GL, Elmore JR, Martinez-Baird J, and Guss AM. Engineered Pseudomonas putida KT2440 co-utilizes galactose and glucose. Biotechnol Biofuels 12, 295 (2019) doi:10.1186/s13068-019-1627-0
- Radivojevic, T., Costello, Z., Workman, K., & Martin, H. G. (2020). A machine learning Automated Recommendation Tool for synthetic biology. Nature Communications, 11(1), 1-14.
- Zhang, Jie, Søren D. Petersen, Tijana Radivojevic, Andrés Ramirez, Andrés Pérez-Manríquez, Eduardo Abeliuk, Benjamín J. Sánchez et al. "Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism." Nature Communications 11, no. 1 (2020): 1-13.





- Ernst Oberortner, Robert Evans, Xianwei Meng, Sangeeta Nath, Hector Plahar, Lisa Simirenko, Angela Tarver, Samuel Deutsch, Nathan J. Hillson, and Jan-Fang Cheng. (2020) "An Integrated Computer-Aided Design and Manufacturing Workflow for Synthetic Biology". In: Chandran S., George K. (eds) DNA Cloning and Assembly. Methods in Molecular Biology, vol 2205. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0908-8\_1
- Gledon Doçi, Lukas Fuchs, Yash Kharbanda, Paul Schickling, Valentin Zulkower, Nathan Hillson, Ernst Oberortner, Neil Swainston, Johannes Kabisch. (2020) "DNA Scanner: a web application for comparing DNA synthesis feasibility, price, and turnaround time across vendors". OUP Synthetic Biology, ysaa011, https://doi.org/10.1093/synbio/ysaa011
- Deng, S., Z. Dai, M. Swita, K.R. Pomraning, B.A. Hofstad, E.A. Panisko, S. Baker and J.K.
   Magnuson 2020. Deletion analysis of the itaconic acid biosynthesis gene cluster components in Aspergillus pseudoterreus ATCC32359. Appl Microbiol Biotechnol 104, 3981-3992.



- Yuqian Gao, Thomas L. Fillmore, Nathalie Munoz Munoz, Gayle J. Bentley, Christopher W. Johnson, Jamie Meadows, Meagan C. Burnet, Anna K. Lipton, Aivett Bilbao Pena, Daniel J. Orton, Young-Mo Kim, Jeremy D. Zucker, Joonhoon Kim, Ronald J. Moore, Errol W. Robinson, Scott E. Baker, Bobbie-Jo M. Webb-Robertson, John M. Gladden, Gregg T. Beckham, Jon K. Magnuson, Kristin E. Burnum-Johnson\*, High-throughput targeted proteomics assays for quantifying large-scale pathway proteins in Pseudomonas putida KT2440, in press at Frontiers Bioeng. Biotechnol.
- Isabel Pardo‡, Ramesh Jha‡, Molly Gaddis, Ryan Bermel, Felicia Bratti, Molly Gaddis, Emily McIntyre, William E. Michener, Ellen L. Neidle, Taraka Dale, Gregg T. Beckham\*, Christopher W. Johnson\*, Gene amplification, laboratory evolution, and biosensor screening reveal MucK as a terephthalic acid transporter in Acinetobacter baylyi ADP1, Metabolic Eng. (2020), 62, 260-274.
- Joshua R. Elmore, Gara N. Dexter, Davinia Salvachúa, Marykate O'Brien, Dawn M. Klingeman, Kent Gorday, Joshua K. Michener, Darren J. Peterson, Gregg T. Beckham, Adam M. Guss\*, Engineering Pseudomonas putida simultaneously catabolizes five major components of lignocellulosic biomass: Glucose, xylose, arabinose, p-coumaric acid, and acetic acid, Metabolic Eng. (2020), 62, 62-71.





- Chris Lawson, Jose Manuel Martí, Tijana Radivojevic, Sai Vamshi R. Jonnalagadda, Reinhard Gentz, Nathan J. Hillson, Sean Peisert, Joonhoon Kim, Blake A. Simmons, Christopher J. Petzold, Steven W. Singer, Aindrila Mukhopadhyay, Deepti Tanjore, Josh Dunn, and Hector Garcia Martin. (2020) "Machine learning for metabolic engineering: A review" Metabolic Engineering https://doi.org/10.1016/j.ymben.2020.10.005
- Riley LA and Guss AM\*. Approaches to genetic tool development for rapid domestication of non-model microorganisms. Biotechnol (2021) 14:30. doi: 10.1186/s13068-020-01872-z.
- Somtirtha Roy, Tijana Radivojevic, Mark Forrer, Jose Manuel Marti, Vamshi Jonnalagadda, Tyler Backman, William Morrell, Hector Plahar, Joonhoon Kim, Nathan Hillson, and Hector Garcia Martin. (2021) "Multiomics Data Collection, Visualization, and Utilization for Guiding Metabolic Engineering". Frontiers in Bioengineering and Biotechnology 9, 45. DOI=10.3389/fbioe.2020.01009



- Pomraning, K., Dai, Z., Munoz, N., Kim, Y., Gao, Y., Deng, S., Kim, J., Hofstad, B., Swita, M., Lemmon, T., Collett, J., Panisko, E., Webb-Robertson, B., Zucker, J., Nicora, C., De Paoli, H., Baker, S., Burnum-Johnson, K., Hillson, N., and Magnuson, J. (2021) Integration of Proteomics and Metabolomics Into the Design, Build, Test, Learn Cycle to Improve 3-Hydroxypropionic Acid Production in Aspergillus pseudoterreus. Frontiers in Bioengineering and Biotechnology.
- Hector A. Plahar, Thomas N. Rich, Stephen D. Lane, William C. Morrell, Leanne Springthorpe, Oge Nnadi, Elena Aravina, Tiffany Dai, Michael J. Fero, Nathan J. Hillson, and Christopher J. Petzold. (2021) BioParts-A Biological Parts Search Portal and Updates to the ICE Parts Registry Software Platform" ACS Synthetic Biology DOI: 10.1021/acssynbio.1c00263"
- Chen Ling, George L. Peabody, Davinia Salvachúa, Young-Mo Kim, Colin M. Kneucker, Michela A. Monninger, Nathalie Munoz, Brenton C. Poirier, Kelsey J. Ramirez, Peter C. St. John, Sean P. Woodworth, Jon K. Magnuson, Kristin E. Burnum-Johnson, Gregg T. Beckham\*, Adam M. Guss,\* Christopher W. Johnson,\* Muconic acid production from glucose and xylose in Pseudomonas putida KT2440 via evolution and metabolic engineering, in review at Nature Comm.





- Nicholas A. Rorrer,‡ Sandra F. Notonier,‡ Brandon C. Knott,‡ Brenna A. Black,‡, Avantika Singh,‡ Scott R. Nicholson,‡ Christopher P. Kinchin, Graham P. Schmidt, Alberta C. Carpenter, Kelsey J. Ramirez, Christopher W. Johnson, Davinia Salvachúa, Michael F. Crowley, Gregg T. Beckham\*, Production of B-ketoadipic acid from glucose in Pseudomonas putida KT2440 for use in performance-advantaged nylons, in review at Cell Reports Phys. Sci.
- Precise genomic riboregulator control of metabolic flux in microbial systems. Naresh Pandey, Steffi A. Davison, Malathy Krishnamurthy, Daniel Trettel, Chien-Chi Lo, Shawn Starkenburg, Katherine L. Wozniak, Theresa Kern, Sean D. Reardon, Clifford J. Unkefer, Scott P. Hennelly and Taraka Dale. Submitted to ACS Synthetic Biology December 2021.
- Chen Y, Kaplan Lease N, Gin JW, Ogorzalek TL, Adams PD, Hillson NJ, et al. (2022) Modular automated bottom-up proteomic sample preparation for high-throughput applications. PLoS ONE 17(2): e0264467. https://doi.org/10.1371/journal.pone.0264467



- Making Security Viral: Shifting Engineering Biology Culture and Publishing Rebecca
  Mackelprang, Katarzyna P. Adamala, Emily R. Aurand, James C. Diggans, Andrew D.
  Ellington, Samuel Weiss Evans, J. L. Clem Fortman, Nathan J. Hillson, Albert W. Hinman,
  Farren J. Isaacs, June I. Medford, Shadi Mamaghani, Tae Seok Moon, Megan J. Palmer, Jean
  Peccoud, Elizabeth A. Vitalis, India Hook-Barnard, and Douglas C. Friedman ACS Synthetic
  Biology 2022 11 (2), 522-527 DOI: 10.1021/acssynbio.1c00324
- Hyun Gyu Lim, Kevin Rychel, Anand V. Sastry, Gayle J. Bentley, Joshua Mueller, Heidi S. Schindel, Peter E. Larsen, Philip D. Laible, Adam M. Guss, Wei Niu, Christopher W. Johnson, Gregg T. Beckham, Adam M. Feist, Bernhard Palsson, Machine-learning from Pseudomonas putida KT2440 transcriptomes reveals its transcriptional regulatory network, Metabolic Eng. (2022) 72, 297-310.
- Lim HG, Rychel K, Sastry AV, Bentley GJ, Mueller J, Schindel HS, Larsen PE, Laible PD, Guss AM, Niu W, Johnson CW, Beckham GT, Feist AM, Palsson BO. Machine-learning from Pseudomonas putida KT2440 transcriptomes reveals its transcriptional regulatory network. Metab Eng. 2022 Jul;72:297-310. doi: 10.1016/j.ymben.2022.04.004. Epub 2022 Apr 27.





- Chen Ling, George L. Peabody, Davinia Salvachúa, Young-Mo Kim, Colin M. Kneucker, Michela A. Monninger, Nathalie Munoz, Brenton C. Poirier, Kelsey J. Ramirez, Peter C. St. John, Sean P. Woodworth, Jon K. Magnuson, Kristin E. Burnum-Johnson, Adam M. Guss,\* Christopher W. Johnson,\* Gregg T. Beckham\*, Muconic acid production from glucose and xylose in Pseudomonas putida KT2440 via evolution and metabolic engineering, Nature Comm. (2022) 13, 4925
- Hector Garcia Martin\*, Tijana Radivojevic, Jeremy Zucker, Kristofer Bouchard, Jess Sustarich, Sean Peisert, Dan Arnold, Nathan Hillson, Gyorgy Babnigg, Jose Manuel Marti, Christopher J. Mungall, Gregg T. Beckham, Lucas Waldburger, James Carothers, ShivShankar Sundaram, Deb Agarwal, Blake A. Simmons, Tyler Backman, Deepanwita Banerjee, Deepti Tanjore, Lavanya Ramakrishnan, Anup Singh, Perspectives for self-driving labs in synthetic biology, submitted to Curr. Opin. Biotech
- N. Pandey, S.A. Davison, M. Krishnamurthy, D.S. Trettel, C. Lo, S. Starkenburg, K.L. Wozniak, T.L. Kern, S.D. Reardon, C.J. Unkefer, S.P. Hennelly and T. Dale. 2022. Precise genomic riboregulator control of metabolic flux in microbial systems. ACS Syn. Biol https://doi.org/10.1021/acssynbio.1c00638





- Pomraning K.R., Z. Dai, N. Munoz Munoz, Y. Kim, Y. Gao, S. Deng, and T.L. Lemmon, et al. 2022. "Itaconic acid production is regulated by laeA in Aspergillus pseudoterreus." Metabolic Engineering Communications
- Kyle R. Pomraning; Shuang Deng; Joonhoon Kim; Kristen B. Campbell; Ana L. Robles; Beth Hofstad; Nathalie Munoz; Yuqian Gao; Teresa Lemmon; Marie S. Swita; Jeremy D. Zucker; Young-Mo Kim; Kristin E. Burnum-Johnson; Jon K. Magnuson; Ziyu Dai. 2022 "Metabolic engineering to improve production of 3-hydroxypropionic acid from corn-stover hydrolysate in Aspergillus species." Metabolic Engineering
- Kevin J. McNaught, Eugene Kuatsjah, Michael Zahn, Érica T. Prates, Huiling Shao, Gayle J. Bentley, Andrew R. Pickford, Josephine N. Gruber, Kelley V. Hestmark, Daniel A. Jacobson, Brenton C. Poirier, Chen Ling, Myrsini San Marchi, William E. Michener, Carrie D. Nicora, Jacob N. Sanders, Caralyn J. Szostkiewicz, Dušan Veličković, Mowei Zhou, Nathalie Munoz, Young-Mo Kim, Jon K. Magnuson, Kristin E. Burnum-Johnson, K.N. Houk, John E. McGeehan, Christopher W. Johnson, Gregg T. Beckham, "Initiation of fatty acid biosynthesis in Pseudomonas putida KT2440", Metabolic Engineering, Volume 76, 2023, Pages 193-203, https://doi.org/10.1016/j.ymben.2023.02.006





- Gregg Beckham, Hybrid biological and catalytic processes to manufacture and recycle plastics,
   Princeton University, November 28th, 2018
- Nathan J. Hillson. "DOE Agile BioFoundry Overview". Invited Talk, SynBioBeta 2018 visit to ESE, Emeryville, CA, October 1, 2018
- Nathan J. Hillson. "Recent developments at the U.S Department of Energy Agile BioFoundry".
   Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 7, 2018
- Nathan J. Hillson. "DIVA (DNA Design, Implementation, Validation Automation) Platform".
   Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 8, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Ginkgo Bioworks, Boston, MA, November 12, 2018; AIChE annual meeting, Pittsburgh, PA, October 31 2018; Thermo Fisher, San Jose, CA, October 19, 2018; DTRA Tech Watch, Ft. Belvoir, VA, October 10, 2018.
- Garcia Martin, H. "A New Approach to Flux Analysis". ABF Annual Meeting, Berkeley CA, September 7, 2018.
- Nathan J. Hillson. "BioDesign Department Overview". Invited Talk, BSE Annual Meeting, Berkeley, CA, January 24, 2019





- Nathan J. Hillson. "Agile BioFoundry Overview". Invited Talk, BETO Peer Review, Denver, CO, March 7, 2019
- Nathan J. Hillson. "Agile BioFoundry DBTL Infrastructure". Invited Talk, BETO Peer Review, Denver, CO, March 7, 2019
- Nathan J. Hillson. "DNA Synthesis Science at the U.S. DOE Joint Genome Institute: Biosecurity Sequence Screening and Broader Aspects Review". Invited Talk, EBRC Spring Retreat 2019, Boston, MA, March 23, 2019
- Nathan J. Hillson. "ABF: plans for the next 3-year cycle". Invited Talk, BETO Quarterly FY19Q2, Washington DC, March 27, 2019
- Jennifer Chiniquy. "Emery Station East DIVA DNA Sequencing". Invited Talk, BSE Annual Meeting, Berkeley, CA, January 24, 2019
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". EmeryStation Campus, Emeryville, CA, February 7th, 2019.
- Garcia Martin, H. "Metabolic modeling, drug synthesis, and their interaction". LBNL Biosciences Experts Advisory Committee, Berkeley, CA, February 8th, 2019.
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Google X, Mountain View, CA, February 14th, 2019.





- Garima Goyal, Z. Costello, J.A. Gutierrez, A. Kang, T.S. Lee, H.G. Martin, and N.J. Hillson.
   "PIACE: Parallel Integration and Chromosomal Expansion of metabolic Pathways". Invited Talk, ACS Conference, Orlando, Florida, April 4, 2019
- Nathan J. Hillson. "DNA synthesis use and biosecurity screening at U.S. DOE projects including the Joint Genome Institute, Joint BioEnergy Institute, and Agile BioFoundry". Invited Talk, Gene Synthesis Governance Meeting, Johns Hopkins Center for Health Security, St. Regis Hotel, Washington, DC, April 9, 2019
- Nathan J. Hillson. "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, SBFC 2019 Session ST-2: Global Research Consortia, Seattle, WA, May 1, 2019
- Nathan J. Hillson. "2019 BETO Merit Review Process". Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. "Machine Learning-assisted MiSeq library loading". Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. "Global Biofoundries Alliance: 2019 Annual Meeting Report Out". Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. "2019 BETO Peer Review Report Out". Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019





- Nathan J. Hillson. "ICE/DIVA, EDD, and ART". Invited Talk, Software for Synthetic Biology Workflows Workshop, SEED 2019, New York, NY, June 27, 2019
- Nathan J. Hillson. "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, JBEI Annual Meeting 2019, Monterrey, CA, May 30, 2019
- Henrique C. De Paoli. "Overview of Synthetic Biology principles". Invited Talk, Xu's Research Group, UC Dept. of Materials Science and Engineering, Berkeley, CA, May 13, 2019.
- Nathan J. Hillson "ABF: CRADA updates and FY20-22". Invited Talk, BETO Conversion Call, July 1, 2019
- Wei Xiong and Nathan Hillson, "Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis". Invited Talk, BETO Conversion Call, July 8, 2019
- Ernst Oberortner, Nathan J. Hillson, and Jan-Fang Cheng. "The Operon Refactoring and Construction Assistant (ORCA): Streamlined gene cluster refactoring". Invited Talk, 11th International Workshop on Bio-Design Automation, University of Cambridge, UK July 9, 2019
- Nathan J. Hillson "ABF Overview, FY19 Update, and FY20-22 Plans". Invited Talk, ABF Annual Meeting, Richland, WA, July 30, 2019
- Nathan J. Hillson "FY22Q4\_DBTL\_AS1: 5x efficiency improvements". Invited Talk, ABF Annual Meeting, Richland, WA, July 31, 2019





- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, VIP Visit - Wendy Pulling | Director of ESG Integration University of California Office of the Chief Investment Officer, Emeryville, CA, Aug 6, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, Tiangong Forum Distinguished Lecture, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 16, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: DBTL Infrastructure". Invited Talk, SynBioYSF 2019, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 17, 2019
- Nathan J. Hillson "U.S. DOE Agile BioFoundry: Overview and Recent Highlights", Invited Talk, Genscript Double Helix Symposium 2019, San Francisco, CA September 30, 2019
- Jennifer Chiniquy "DIVA DNA Sequencing". Invited Talk, 2019 ABF All Hands Meeting, Pacific Northwest National Laboratory, Richland, WA, July 30, 2019.
- Tijana Radivojevic, "Automatic Recommendation Tool", Invited Talk, ABF Annual Meeting 2019, Richland, WA, July 30, 2019
- Nurgul Kaplan."Automated DNA Construction: "from j5 protocol design to Laboratory Robotics" Invited Talk, ABF Annual Meeting 2019, Pasco, WA, July 30, 2019





- Nathan J. Hillson "U.S. DOE Agile BioFoundry: Organization and Capabilities", Invited Talk, ABF Industry Day 2019, Emeryville, CA October 4, 2019
- Garcia Martin, H. "Machine Learning, Synthetic Biology and Automation: Engineering Life for the Benefit of Society". NERSC data seminar, Berkeley CA, November 1st, 2019.
- Garcia Martin, H. "ART: a machine learning Automated Recommendation Tool for guiding synthetic biology". Al4Synbio Symposium, Arlington VA, November 8th, 2019.
- Garcia Martin, H. "Opportunities in the intersection of:Artificial Intelligence & Synthetic Biology & Automation". Army Science Planning and Strategy Meeting, Burlington MA, November 13th, 2019.
- Nathan J. Hillson "ABF: CRADA updates and FY20-22". Invited Talk, BETO Conversion Call, July 1, 2019
- Wei Xiong and Nathan Hillson, "Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis". Invited Talk, BETO Conversion Call, July 8, 2019
- Ernst Oberortner, Nathan J. Hillson, and Jan-Fang Cheng. "The Operon Refactoring and Construction Assistant (ORCA): Streamlined gene cluster refactoring". Invited Talk, 11th International Workshop on Bio-Design Automation, University of Cambridge, UK July 9, 2019





- Nathan J. Hillson "ABF Overview, FY19 Update, and FY20-22 Plans". Invited Talk, ABF Annual Meeting, Richland, WA, July 30, 2019
- Nathan J. Hillson "FY22Q4\_DBTL\_AS1: 5x efficiency improvements". Invited Talk, ABF Annual Meeting, Richland, WA, July 31, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, VIP Visit - Wendy Pulling | Director of ESG Integration University of California Office of the Chief Investment Officer, Emeryville, CA, Aug 6, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, Tiangong Forum Distinguished Lecture, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 16, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: DBTL Infrastructure". Invited Talk, SynBioYSF 2019, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 17, 2019
- Nathan J. Hillson "U.S. DOE Agile BioFoundry: Overview and Recent Highlights", Invited Talk, Genscript Double Helix Symposium 2019, San Francisco, CA September 30, 2019
- Jennifer Chiniquy "DIVA DNA Sequencing". Invited Talk, 2019 ABF All Hands Meeting, Pacific Northwest National Laboratory, Richland, WA, July 30, 2019.





- Tijana Radivojevic, "Automatic Recommendation Tool", Invited Talk, ABF Annual Meeting 2019, Richland, WA, July 30, 2019
- Nurgul Kaplan."Automated DNA Construction: "from j5 protocol design to Laboratory Robotics"
   Invited Talk, ABF Annual Meeting 2019, Pasco, WA, July 30, 2019
- ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology",
   Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- Garcia Martin, H. "Opportunities in the intersection of machine learning, synthetic biology, and automation". ABLC 2020, Virtual meeting, July 10th, 2020.
- Garcia Martin, H. "Leveraging machine learning and automation to make synthetic biology predictable". SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020
- Nathan J. Hillson, "Perspectives from the U.S. DOE Agile BioFoundry", OECD BNCT Virtual Workshop, Session 1: Biofoundries and COVID-19, via Zoom, July 29, 2020
- Nathan J. Hillson, "DIVA, EDD, and ART: Software spanning the Design/Build/Test/Learn cycle", invited talk, COMBINE 2020, via Zoom, October 5, 2020





- Nathan J. Hillson, "DIVA, EDD, and ART: Software spanning the Design/Build/Test/Learn cycle", invited talk, SPARC Workshop (IIT Kharagpur) 2020, via Zoom, October 19, 2020
- Invited talk: Guss AM. "Domestication of non-model microbes for the production of renewable fuels and chemicals" (2021) The Metabolic Engineering Virtual Seminar Series at the University of Texas-Austin. Virtual.
- Garcia Martin, H. "Leveraging machine learning and automation to make bioengineering predictive". The Metabolic Engineering Virtual Seminar, UT Austin, February 12th, 2021.
- Garcia Martin, H. "Machine Learning for Bioengineering". JBEI Board of Directors, March 12th, 2021.
- Nathan J. Hillson, "ABF Overview", invited talk, BETO Peer Review 2021, via Zoom, March 9, 2021
- Nathan J. Hillson, "ABF DBTL Infrastructure", invited talk, BETO Peer Review 2021, via Zoom, March 9, 2021
- Nathan J. Hillson, "ABF Overview", invited talk, BSA ExComm, via Zoom, March 12, 2021
- Guss AM. "Synthetic biology and metabolic engineering of non-model microbes for the production of renewable fuels and chemicals" Seminar at Purdue University Northwest. April 16, 2021.





- Radivojevic, T., "Automated Recommendation Tool (ART): Design of Experiments via Probabilistic Predictive Modeling", Autonomous Discovery in Science and Engineering Workshop, April 21, 2021(Invited Tutorial)
- Radivojevic, T., "Guiding synthetic biology via machine learning", Symposium on Biomaterials,
   Fuels and Chemicals (SBF): Speeding up synthetic biology, April 27, 2021
- Radivojevic, T., "Guiding synthetic biology via Automated Recommendation Tool (ART)", Synthetic Biology: Engineering, Evolution & Design (SEED) 2021, Computation, Artificial Intelligence, and Machine Learning for Biological Design Session, June 17, 2021 (Invited Speaker)
- Guss AM. "Domestication of non-model microbes for the production of renewable fuels and chemicals" Student-invited seminar at North Carolina State University. September 27, 2021.
- Radivojevic, T. "Guiding synthetic biology via machine learning and multi-omics technologies", IWBDA 2021, 13th International Workshop on Bio-Design Automation, September 20, 2021 (virtual)
- Hillson, Nathan J. "Session Introduction", Metabolic Engineering 14, Block 8: Session Biofoundries, (Virtual via Remo), July 15, 2021





- Hillson, Nathan J. "Updates on DOE assets including Agile BioFoundry, Joint BioEnergy Institute, and Joint Genome Institute", Digital Biology Engineering Meeting (Air Force) via zoom, September 23, 2021
- Garcia Martin, H. "Machine Learning Tools Can Make Synthetic Biology Predictable". SIMB, August 9th, 2021.
- Garcia Martin, H. "Leveraging machine learning and automation to make bioengineering predictable". Indo-US Workshop on Application of Data Science in Biological Systems, September 7th, 2021.
- Garcia Martin, H. "Leveraging machine learning and automation to make bioengineering predictable". Air Force Research Laboratory MIrACLE Forum, September 10th, 2021.
- Garcia Martin, H. "Challenges and opportunities in high-throughput data synthesis". NSF
  Challenges and Opportunities in Synthesizing Massively Parallel Assays and High-Throughput
  Datasets Workshop, September 17th, 2021.
- Garcia Martin, H. "Guiding metabolic engineering via kinetic deep learning and multi-omics".
   Quantitative Modelling of Cell Metabolism Conference, September 21th, 2021.
- Davison, S. Reprogramming Microbes for Biomanufacturing, Science in 3 at Los Alamos National Laboratory, September 2021





- Wozniak, K. Engineering a Sustainable Future: Fine-tuning Gene Expression for Biomanufacturing, Science in 3 at Los Alamos National Laboratory, September 2021
- Davison, S. Fine-tuning gene expression in bioproduction pathways in diverse bacterial hosts,
   SIMB Annual Meeting, August.
- Wozniak, K. Targeting global regulatory responses using precise and programmable cisriboregulators SIMB Annual Meeting, August
- Adam Guss. "Genetic tools and microbial engineering for biological production of sustainable fuels and chemicals" Presented to Weekly Seminar for DOE CCI/SULI Students. October 27, 2021
- Adam Guss. "Domestication of diverse non-model microbes for plastics upcycling and sustainable fuel and chemical production" Biological Sciences Departmental Seminar, Michigan Technical University. Oct 28, 2021.
- Garcia Martin, H. "Guiding metabolic engineering via kinetic deep learning and multi-omics".
   Quantitative Modelling of Cell Metabolism Conference, October 20th, 2021.
- Garcia Martin, H. "Machine learning for industrial biotechnology". Delft Advanced Course Integrated Multi-Omics approaches for Improvement of Industrial Microbes, November 5th, 2021.





- Hillson, Nathan J. "Opportunities for CABBI collaboration with JBEI (and other DOE assets) in laboratory operations and data management infrastructure", CABBI seminar, via zoom, October 19, 2021
- Hillson, Nathan J. "Automation in Biotechnology: Challenges and Opportunities", Panelist at SENAI CETIQT (Brazil), via zoom, October 29, 2021
- Hillson, Nathan J. "ABF Overview and Capabilities", ABF Industry Day (via zoom), November 19, 2021
- Hector A Plahar, Stephen D. Lane, William C Morrell, Nathan J. Hillson and Christopher J. Petzold. "A Biological Parts Search Portal and Updates to the ICE Parts Registry Platform", JBEI Annual DOE Review, Dec 6-8, 2021
- Tijana Radivojevic, "Creating a Machine Learning Chassis to Maximize the Efficiency of the DBTL Cycle in Synthetic Biology", Synthetic Biology-Based Therapeutics Summit, December 9, 2021 (virtual)
- Hillson, Nathan J. "Overview and capabilities of the ABF and opportunities for collaboration with CSU", Colorado State Seminar Series (via zoom), February 3, 2022
- Hillson, Nathan J. "ABF prospective collaboration with other BETO consortia and projects", BETO Conversion Call (via MS Teams), March 7, 2022





- Bilbao, A. "Investigating deep learning approaches to advance data processing in Liquid Chromatography, Ion Mobility and Data-Independent Acquisition Mass Spectrometry Omics".
   70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 9th, 2022.
- Dai Z., K.R. Pomraning, S. Deng, J. Kim, K.B. Campbell, A.L. Robles, and B.A. Hofstad, et al. 05/02/2022. "Assessment and Optimization of 3-hydroxypropionic acid production in industrial filamentous fungus-Aspergillus species." Presented by Z. Dai at 44th Symposium on Biotechnology for Fuels and Chemicals (SBFC 2022), New Orleans, Louisiana
- Adam Guss. "High efficiency DNA integration in diverse non-model microbes for rapid tool and pathway prototyping" SIMB SBFC. New Orleans, LA, May 3, 2022
- Magnuson, J.K.; Beckham, G.T.; Gladden, J.M.; Dale, T.; Guss, A.M.; Laible, P.; Hillson, N.J. 05/03/2022. "The Agile BioFoundry" Presented by Jon Magnuson at the 44th Symposium on Biotechnology for Fuels and Chemicals (SBFC 2022), New Orleans, Louisiana.
- Poirier, B.C. et al. "Mechanical cell disruption enhances the extraction of metabolites from bacterial and fungal species commonly used in metabolic engineering". 70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 5-9, 2022.





- Poirier B.C. et al. "Behavior of lactam molecules during aqueous sample preparation and mass spectrometry analysis". 70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 5-9, 2022.
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", ABF Webinar (via zoom) April 29, 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", Lessafre visit to ESE (via zoom) May 6, 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry" (Keynote Presentation) Bioindustry 4.0 – Synthetic Biology & Biofoundry, CHEY Institute – Scientific Innovation Series (via zoom) 12 May 17 (PDT) 18 (KST), 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", Wageningen University visit to ESE (via zoom) May 31, 2022
- Hillson, Nathan J. "ABF overview and DBTL Infrastructure", BETO Conversion Call (via MS Teams), June 13, 2022
- Nathan Hillson. "ABF Decarbonization Efforts", Invited Talk, LBL Carbon Negative Initiative –
   Biological Applications Lightning Talk Session (via zoom) 6/27/2022





- Radivojevic, Tijana. "Guiding synthetic biology via machine learning", Boston University, April 14, 2022, Guest lecture
- Deng S., J. Kim, K.R. Pomraning, Z. Dai, Y. Gao, N. Munoz Munoz, and Y. Kim, et al. 08/07/2022. "Identification of a specific exporter that enables high production of aconitic acid in A. pseudoterreus ATCC32359." Presented by S. Deng at SIMB annual meeting, San Francisco, California.
- Pomraning K.R., Z. Dai, S. Deng, N. Munoz Munoz, Y. Kim, B.A. Hofstad, and Y. Gao, et al. 08/10/2022. "Bioconversion of lignocellulosic feedstocks to 3-hydroxypropionic acid using acidophilic fungi." Presented by K.R. Pomraning at Society for Industrial Microbiology and Biotechnology, San Francisco, California.
- Jha RK. High throughput test tools for industrially relevant microbial chassis, SIMB 2022, San Francisco, August 2022 (invited talk).
- Adam Guss. "Using synthetic biology to solve challenges in plastic waste and renewable chemical production". Biological Sciences Departmental Seminar, Missouri S&T, Rolla, MO. September 27, 2022.
- Nathan Hillson. "Agile BioFoundry Connections with NSF, MSRDC, and Beyond", Invited Talk, LBL Biosciences Expert Advisory Committee: connections beyond Biosciences July 8, 2022





- Nathan Hillson. "Biosecurity concern anecdote", Screening Tools for Genome Engineering and Genome Editing (Inscripta-funded; Sarah Carter organized), via MS Teams, 9/9/2022
- Nathan Hillson. "Webinar Agenda and ABF Overview", ABF Webinar: Cutting-Edge
   Technologies for Accelerating Bioproduct Development at the Agile BioFoundry, 9/22/20222
- Garcia Martin, H. "Machine Learning and Automation for Predictive Synthetic Biology". Basque Center for Applied Mathematics, Bilbao, Spain, July 15th, 2022.



# License partners and patent applications

#### **Technologies licensed**

- Bioproduction of limonene from syngas
- · Method to produce branched chain polyhydroxyalkanoates and branched chain 3-hydroxyacids

#### **Provisional Patents**

- ROI-18-92 U.S. provisional patent applications 63/163,518 63/321,207 63/479,918, not published
- ROI-21-104 U.S. provisional patent application 63/321,332
- ROI-21-63 U.S. provisional patent applications 63/163,518 63/321,207 63/479,918, not published

#### **Patent Applications**

- Post-transcriptional genome regulation in bacteria with next generation CRISPR-Cas tools
- · Terephthalate biosensor and applications thereof
- · Mutant transporters for bacterial uptake of terephthalic acid
- Alleviating the bottleneck in enzyme evolution and pathway optimization using novel biosensors (Disclosure Title) Modified Biosensors and Biocatalysts and Methods of Use (Application Title)
- Mutant transporters for bacterial uptake of terephthalic acid
- ART: A machine learning Automated Recommendation Tool for guiding synthetic biology





# Patent applications

#### **Patent Applications (cont.)**

- A Generative Model for Protein Sequences for the Purpose of Protein Design or Phenotypic Inference
- Predicting Metabolic Pathway Dynamics from Time Series Multiomics Data Using Machine Learning Techniques
- Use of Statistical Learn Approaches to Predict Next Generation Sequencing Subsequence Depth of Coverage
- Mutant transporters for bacterial update of terepthalic acid
- Method and strain for sugar conversion
- Engineered Microorganisms for the Production of Intermediates and Final Products (1st)
- Engineered Microorganisms for the Production of Intermediates and Final Products (2<sup>nd</sup>)
- Production of organic acids from Aspergillus pseduoterreus cadA deletion strain (1st)
- Production of organic acids from Aspergillus pseduoterreus cadA deletion strain (2<sup>nd</sup>)
- Genetically engineering an industrial filamentous fungus Aspergillus niger for 3hydroxypropionic acid production
- A specific exporter responsible for aconitic acid high production in Aspergillus pseduoterreus





#### Records of invention

#### **Records of Invention**

- Bioproduction of limonene from syngas
- Mutant transporters for bacterial update of terepthalic acid
- Method to produce branched chain polyhydroxyalkanoates and branched chain 3hydroxyacids
- A genetic circuit to reduce cell-to-cell production heterogeneity
- High yield conversion of D-xylose to D-arabitol in R. toruloides
- Manipulation of tRNA thiolation gene ncs2 for enhanced production of fatty-acyl-CoA derived chemicals in R. toruloides
- Efficient production of cis, cis-muconic acid from mixed substrates of glucose, D-xylose and L-arabinose
- Whole cell biosensors for industrially relevant polymers precursors
- Engineered Microorganisms for the Production of Intermediates and Final Products
- Method and strain for sugar conversion





## Software disclosures

#### **Software Disclosures**

- Automated Recommendation Tool (ART) v2.0
- Kinetic Learning v0.1
- Automated Recommendation Tool (ART): v1.0
- PIACE: Parallel Integration and Chromosomal Expansion of Metabolic Pathways
- OMG, Omics Mock Generator Library: v0.1.1
- Fermentation Data Processing
- Fermentation Data Manipulation and Analysis Once imported
- DIVA/Device Editor 3.1
- DIVA/Device Editor (DIVA) v6.0.0



